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Effect of *Mucuna pruriens* and *Glycyrrhiza glabra* on locomotor behavior of *Drosophila melanogaster* (Oregon K strain) and vestigial wing mutant flies

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The circadian rhythms are ubiquitous and confer an adaptive advantage to an individual/population by synchronising its daily activity to its surroundings. Circadian rhythms are controlled by self-sustained oscillator and they continue to oscillate in constant condition. Locomotor activity is one such robust self sustained oscillation. It is essential/crucial for an organism's survival such as feeding, mate choice, escape from the predator, etc. The present investigations were made to understand the effect of *Mucuna pruriens* and *Glycyrrhiza glabra* on locomotor behaviour of the *Drosophila melanogaster* (Oregon K strain) and vestigial wing mutant flies. Locomotor activity rhythms were assayed in *D. melanogaster* and vestigial wing mutant flies using *Drosophila* activity monitor and the amount of activity was measured. The results revealed that the *Mucuna pruriens* and *Glycyrrhiza glabra* treated flies showed increased activity when compared to control flies, between treated groups, higher concentration treated flies showed maximum activity than lower concentration treated flies. Statistical analysis showed that there is a significant difference in the locomotor activity among control and treated groups of *D. melanogaster* and vestigial wing mutant of *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract.

Keywords: Circadian, Oscillation, Self-sustained, Synchronising, Ubiquitous

Ayurveda often has been considered as one of the earliest medicinal system in the world[1]. Many of the ayurvedic drugs are manufactured as various herbal preparations. *Mucuna pruriens* and *Glycyrrhiza glabra* are two such plants which are used mainly as aphrodisiacs in addition to several other purposes. Of these *Mucuna pruriens* belongs to Family Fabaceae and sub family Papilionaceae. It is an annual tropical legume and grows in southern and Southeast Asian regions[2]. *Mucuna pruriens* is used as aphrodisiac for male virility and a nerve tonic for nervous system disorders. The seeds of *Mucuna pruriens* have proved to be effective in reducing the effects of Parkinson's disease[3]. It also has antineoplastic, antioxidant, antidiabetic, antimicrobial, analgesic and anti-inflammatory activities[4]. The powder of the seed helps in reducing stress, increases secretion of semen and acts as restoring and invigorating tonic or an aphrodisiac[5]. *Glycyrrhiza glabra* is another plant which also has aphrodisiac property is commonly known as liquorice and sweet wood. It belongs to family leguminaceae and sub family Faboideae. It is native of the Mediterranean and certain areas of Asia. Liquorice is used as laxative demulcent, antitussive (capable of relieving or suppressing cough) and sweetener. In modern medicine, liquorice extracts are used to cure cold. It also has therapeutic benefit against other viruses, including cytomegalovirus, Human immunodeficiency viruses and Herpes simplex. The study was intended to analyse the effect of extract of *Mucuna pruriens* seed and *Glycyrrhiza glabra* root on locomotor behaviour of *D. melanogaster* and vestigial wing mutant flies. *D. melanogaster* has been used as the best test system in mutagenic and genotoxic studies, screening various drugs and pharmaceuticals for their effect, the protocols such as dominant lethal test, sex lined recessive lethal test, autosomal recessive lethal test etc have been developed which makes this fly suitable for the analysis of various drugs and chemicals. Vestigial wing mutant used in the present study was one of the first mutants with a wing phenotype to be characterised in *D. melanogaster*[6].

Materials and Methods

Flies stocks used for the present analysis were *D. melanogaster* (Oregan K strain) and vestigial wing mutants. These flies were obtained from *Drosophila* Stock Centre, Department of Zoology, University of Mysore, Mysore, Karnataka, and maintained under laboratory condition at a constant temperature of 22±1°C with 75% relative humidity. Flies were cultured in a standard soji agar medium fed with yeast granules[7].

Preparation of plant extracts

Mucuna pruriens seed and *Glycyrrhiza glabra* root were collected from a local panchasara store, Mysore,

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Karnataka. They were shade dried and milled into coarse powder using a mechanical grinder. The coarse powder was extracted with water by decoction in a round bottom flask. The extract was evaporated into a semi-solid mass, which was dried and stored for future use and the aqueous extract of *Glycyrrhizia glabra* root and *Mucuna pruriens* seed were used for the present study. In case of treated groups, two concentrations of *Glycyrrhiza glabra* root, namely 1.0mg/100 mL (0.01mg/ mL) and 2.0mg/100 mL (0.02mg/ mL) mixed in wheat cream agar medium labeled as (T_1 and T_2), were used, respectively. In order to fix the concentrations of root extract of *Glycyrrihiza glabra*, LC_{50} was determined using the log dose probit method. The lethal concentration of the extract is 3.0mg/100 mL (0.03mg/ mL). The sub lethal (effective concentration) concentrations are T_1 and T_2 as mentioned above. Similarly, for treated groups of the *Mucuna pruriens* seed extract, two concentrations were taken, viz, 2.0mg/100 mL (0.02mg/ mL) and 2.5mg/100mL (0.025mg/mL) mixed in wheat cream agar medium labeled as T_1 and T_2, were used, respectively. In order to fix the concentrations of the seed extract of *Mucuna pruriens*, LC_{50} was determined using the log dose probit method. The lethal concentration for this test is 4.0mg/100mL (0.04mg/mL). The sub lethal (effective concentration) concentrations are T_1 and T_2 as mentioned above. Eight mL of food media containing different concentrations of the extracts were added to individual glass vials. The flies were allowed to feed on these media. The flies were cultured on 8mL of normal medium simultaneously served as a control.

Locomotor activity

Locomotor activity was analysed by *Drosophila* Activity Monitor (Fig. 1) (Trikinetics, Inc., Waltham, MA)[8]. The activity of an individual fly was monitored by placing the fly into a glass tube (65mm length × 5mm diameter). The tube was inserted into a ring detector, in which one end of the tube was provided with normal culture medium (2g agar + 2g sucrose in 100mL distilled water) and the other end was plugged with cotton. The tube was placed in the path of an infrared beam. Likewise, the activities of 32 flies were recorded at a time. The culture medium was replenished once in 2 days. Every interruption of an infrared beam by the fly movement triggered an all-or-none electric signal that was amplified, counted, accumulated and then registered. Every time the fly moves is referred as bout and was summed at 1h intervals known as bins (1h = 1bin). Such records were done in 25 replicates for 12 days continuously without interruption. The activity performed by the flies in the first two days was ignored because it marked the period of acclimatisation. The locomotor activity data was analysed by using ANOVA in SPSS software version 14.0.

Fig. 1 — *Drosophila* Activity Monitor

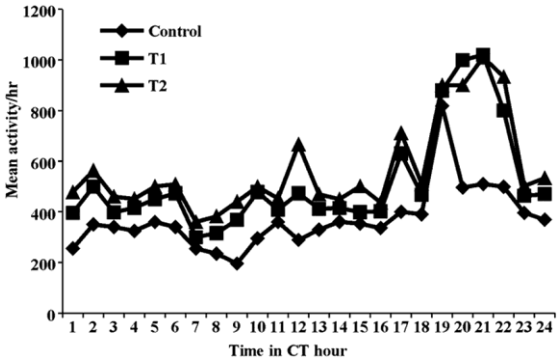

Fig. 2 — Locomotor activity of *Drosophila melanogaster* in control and treated with *Glycyrrhiza glabra* root extract

Results and Discussion

Locomotor activity of *D. melanogaster* in control and treated groups of *Glycyrrhiza glabra* root extract (Fig. 2) has increased activity in treated groups when compared to control flies, between treated groups, higher concentration treated flies i.e. 2.0mg/100mL (0.02mg/mL) i.e. treated 2 (T2) showed maximum activity than lower concentration treated flies i.e. 1.0mg/100 mL (0.01mg/ mL) i.e. treated 1 (T1). Statistical analysis showed that there is a significant difference in the locomotor activity among control and treated groups of *D. melanogaster* ($F=8.413$, $P<0.05$) (Table 1), whereas vestigial wing mutants treated with *Glycyrrhiza glabra* root extract (Fig. 3)

Table 1 — Mean ± Standard Error of Locomotor activity of *Drosophila melanogaster* and vestigial wing mutant in control, *Glycyrrhiza glabra* root extract and *Mucuna pruriens* seed extract treated groups

		Locomotor activity	Mean±Standard Error	F	Significance
Control and *Glycyrrhiza glabra* root extract treated groups	*Drosophila melanogaster*	Control	369.08±25.16	8.413	0.001*
		T1	513.87±41.17		
		T2	567.37±37.74		
	Vestigial wing mutant	Control	209.45±13.46	11.109	0.001*
		T1	243.37±13.63		
		T2	319.54±22.74		
Control and *Mucuna pruriens* seed extract treated groups	*Drosophila melanogaster*	Control	369.16±23.80	8.809	0.001*
		T1	525.08±44.38		
		T2	591.25±43.48		
	Vestigial wing mutant	Control	187.50±12.96	39.432	0.001*
		T1	261.33±14.45		
		T2	361.00±14.13		

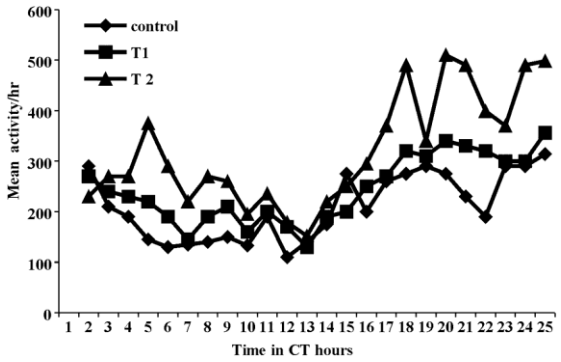

Fig. 3 — Locomotor activity of vestigial wing mutant in control and treated with *Glycyrrhiza glabra* root extract

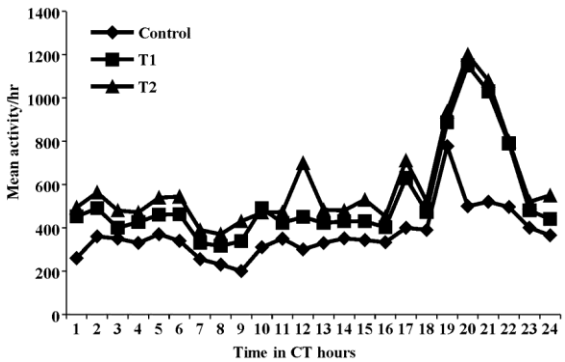

Fig. 4 — Locomotor activity of *Drosophila melanogaster* in control and treated with *Mucuna pruriens* seed extract

showed enhanced locomotor activity in treated groups when compared to control (F=11.10, *P*<0. 05) (Table 1).

D. melanogaster flies treated with *Mucuna pruriens* seed extract (Fig. 4) also has maximum locomotor activity than control flies. In higher concentration 2.5mg/100mL (0.025mg/mL) of *Mucuna pruriens* treated groups, there was maximum activity when compared to lower concentration 2.0mg/100mL (0.02mg/mL). Statistical analysis showed that there is a significant difference in the locomotor activity among control and treated groups of *D. melanogaster* (F=8.809, *P*<0.05) (Table 1) and it was observed that there was increased in locomotor activity of vestigial wing mutant treated with *Mucuna pruriens* seed extract compared to control (Fig. 5). Statistical analysis showed that there is a significant

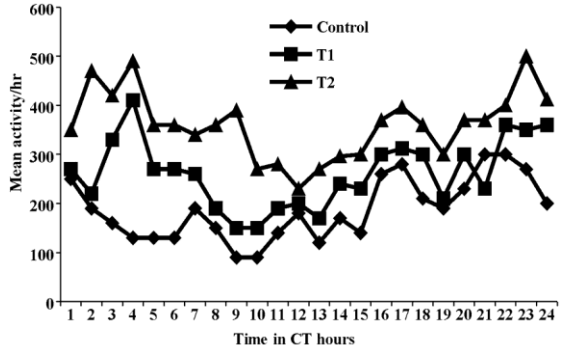

Fig. 5 — Locomotor activity of vestigial wing mutant in control and treated with *Mucuna pruriens* seed extract

difference in the locomotor activity among control and treated groups (F=39.432, *P*<0.05) (Table 1).

Locomotor activity of *D. melanogaster* treated with *Mucuna pruriens* seed extract in percentage (Table 2)

Table 2 — Comparison of Locomotor activity of *Drosophila melanogaster* treated with *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract in percentage

	Mucuna pruriens			*Glycyrrhiza glabra*		
Circadian Time (hours)	Control	T1	T2	Control	T1	T2
1	260	452	494	255	396	478
2	360	490	563	350	500	563
3	350	400	480	340	398	460
4	330	426	471	325	416	451
5	370	460	539	360	450	500
6	340	463	544	340	473	509
7	255	330	390	255	300	360
8	230	316	370	235	316	382
9	200	338	430	196	368	440
10	310	490	470	295	478	499
11	350	423	473	360	409	453
12	300	450	700	289	473	666
13	330	422	483	330	412	469
14	351	430	480	361	415	450
15	343	430	530	353	399	501
16	333	402	452	336	402	437
17	400	630	711	400	630	711
18	390	473	523	390	467	511
19	777	887	937	818	879	899
20	500	1150	1200	496	999	900
21	520	1030	1080	510	1020	1010
22	496	790	800	499	800	933
23	400	480	520	396	463	502
24	365	440	550	369	470	533
Sum	8860	12602	14190	8858	12333	13617
Percentage	24	35	39	24	34	38

showed that there is 11% and 15% increase in locomotor activity of *D. melanogaster* T1 and T2 groups respectively. There is 4% difference was observed between T1 and T2. Comparison of locomotor activity of *D. melanogaster* treated with *Mucuna pruriens* seed extract showed there is increase in locomotor activity as the concentration increases compared to control (Table 2). Pearson's correlation between two plant extracts show significant result in *D. melanogaster* (Table 4).

Locomotor activity of vestigial wing mutant flies in percentage (Table 3) showed there is 21.50% increase in locomotor activity in T2 flies and 12.5% increase in T1 flies. Between T1 and T2 groups 9% difference was observed. Comparison of locomotor activity of vestigial wing mutant flies treated with *Mucuna pruriens* seed extract shows as concentration increases there is increase in locomotor activity (Table 3). Pearson's correlation between two plant extracts is significance at various level (Table 4).

Comparison of locomotor activity of *D. Melanogaster* treated with *Glycyrrhiza glabra* root

Table 3 — Comparison of Locomotor activity of vestigial wing mutant treated with *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract in percentage

	Mucuna pruriens.			*Glycyrrhiza glabra*.		
Circadian Time (h)	Control	T1	T2	control	T1	T 2
1	250	270	350	290	270	230
2	190	220	470	210	240	270
3	160	330	420	190	230	270
4	130	410	490	145	220	375
5	130	270	360	130	190	290
6	130	270	360	135	145	220
7	190	260	340	140	190	270
8	150	190	360	150	210	260
9	90	150	390	133	160	195
10	90	150	270	190	200	236
11	140	190	280	110	170	179
12	180	200	230	140	130	152
13	120	170	270	175	190	220
14	170	240	296	275	200	250
15	140	230	300	200	250	295
16	260	300	370	260	270	370

(*Contd*.)

Table 3 — Comparison of Locomotor activity of vestigial wing mutant treated with *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract in percentage (*Contd*.)

	Mucuna pruriens.			*Glycyrrhiza glabra*.		
Circadian Time (h)	Control	T1	T2	control	T1	T 2
17	280	312	396	275	320	490
18	210	300	360	290	310	340
19	190	210	300	275	340	510
20	230	300	370	230	330	490
21	300	230	370	190	320	399
22	300	360	400	290	300	370
23	270	350	500	290	300	490
24	200	360	412	314	356	498
Sum	4500	6272	8664	5027	5841	7669
Percentage	23	32	44.50	27.10	31.50	41.30

extract in percentage (Table 2) showed that there is 10% and 14% increase in locomotor activity of *D. melanogaster* T1 and T2 group respectively. A difference of 4% was observed between T1 and T2 groups. Comparison of locomotor activity of *D. melanogaster* treated with *Glycyrrhiza glabra* root extract has increased locomotor activity as the concentration increases compared to control flies (Table 2). Pearson's correlation of *D. melanogaster* between two plant extracts show significance at various levels (Table 4).

Vestigial wing mutant flies have increased locomotor activity i.e., 14.2% and 4.4% respectively in T2 and T1 groups treated with *Glycyrrhiza glabra* root extract (Table 3). 9.8% of difference was seen between T1 and T2 groups. Comparison of locomotor activity of vestigial wing mutant flies treated with *Glycyrrhiza glabra* root extract showed increased locomotor activity compare to control group (Table 3). Pearson's correlation of vestigial wing mutant flies between two plant extracts show significance at various level (Table 4).

Locomotion is an integral component of any animal's behaviour. It is very much essential for localisation of food and mates, to escape from predators, defense of territory and even often response to stress. It is a complex behavior sensitive to environmental changes[9,10]. *D. melanogaster* traditionally and currently serves as a model organism for virtually all aspects of biology, but especially for genetics and development[11]. Evidence suggests that as many as 75% of human disease genes are conserved in *Drosophila*[12] furthermore 68% of human disease gene have homologous in *Drosophila*[13]. Many scientists have used *D. melanogaster* as model organism in testing various plant extract such as resveratrol which prolongs the lifespan of worm *Caenorhabaditis elegans* and *D. melanogaster*[14]. In the present study locomotor rhythm was analysed in these flies by treating them with different plant extracts. The locomotor rhythm of *D. melanogaster* in control showed 2 peaks, one is the weak morning peak and the strong evening peak at 19h. The activity level is very much reduced in the noon. The locomotor rhythm of the flies treated with two plant extracts under study has showed no difference but the enhanced activity is observed. The strong evening peak with a weak morning peak was observed for both the plant extract treated flies. Enhanced morning peak was observed. The period calculated for all the treated groups remain same i.e. approximately 24h. The *Glycyrrhiza glabra* treated flies showed highest activity when compared to control flies. The amount of activity is increased at different hours of the day. Flies showed increased activity in *Mucuna pruriens* seed extract T1 and T2 groups when compared to control flies. The activity levels of T1 and T2 is enhanced to 19h, 20h, and 21h of the day with strong evening peak. It is also observed that noon activity also increasedcompared to control group.

On the other hand vestigial (*Vg*) mutations in *Drosophila* cause deletions of parts of the wing. The size and nature of the deletions depend upon the particular *Vg* allele[15,16]. Vestigial expression is evident in thoracic and abdominal segments, in the embryonic primordial of the wing and haltere discs, in discrete cells in the ventral nerve cord, and possibly in progenitors of sense organs of the peripheral nerve cord[17]. There are no phenotypes associated with vestigial mutation, either in segmentation or neural development, but mutant flies manifest a complete lack of balance organs of the third thoracic segment, as well as a lack of development of wings and halteres. Due to inability of these flies to fly, they were selected to test the effect of plant extracts on the locomotion. The vestigial flies showed increased activity throughout the day without affecting the morning and evening peak. Compared to *D. melanogaster*, vestigial activity is increased at different hours of the day. The peak activity is observed at 14h, 16h, 17h, 18h, 19h, 21h and 24h in T1 group, from 14h - 21h in T2 group compared to control group. The activity level prolonged up to four hours in both T1 and T2. Totally two peaks were observed one weak morning peak and strong evening

Table 4 — Pearson's Correlation of Locomotor activity of *Drosophila melanogaster* and vestigial wing mutant treated with *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract

		Mucuna pruriens						*Glycyrrhiza glabra*					
		Drosophila melanogaster			vestigial wing mutant			*Drosophila melanogaster*			vestigial wing mutant		
		Control	T1	T2	Control	T1	T2	Control	T1	T2	Control	T1	T2
Mucuna pruriens	Control	1											
	T1	0.554	1		1								
	T2	0.531	0.791	1	0.501	1							
Glycyrrhia glabra	Control	0.528	0.762	0.975	0.344	0.689	1	1					
	T1	0.530	0.997	0.765	0.661	0.462	0.264	0.732	1		1		
	T2	0.561	0.818	0.988	0.712	0.498	0.387	0.971	0.786	1	0.815	1	
		0.564	0.794	0.935	0.660	0.606	0.481	0.948	0.766	0.967	1	0.724	0.920	1

[Values are significant at 0.05 levels; Value of r is 0.997 of *Drosophila melanogaster* which is nearly equal to 1 and r value of vestigial wing mutant treated flies is 0.991 which is nearly equal to 1 which shows positive correlation.]

peak with reduced activity level during noon in the control group. Whereas, amount of activity increased with two strong peak both evening and morning in treated groups. The activity level was enhanced during the peak hours upto 4 hours duration. Vestigial wing mutant treated with *Mucuna pruriens* has showed an increased activity level similar to *Glycyrrhiza glabra.* The amount of activity at different hours of the day is more at 4h (strong morning peak), 22h, 23h and 24h strong evening peak. Vestigial wing mutant exhibits a strong morning and evening peak, yet the period has not altered it is approximately 24h similar to control group. The performance of the flies in *Mucuna pruriens* is more compared to *Glycyrrhiza glabra*. The period is 24h for all the groups. Hence the rhythm is not altered by the treatment however the amount of activity is enhanced. It showed positive correlation when the Pearson's correlation is done. The study has shown significant increase in the locomotor activity of *D. melanogaster* and vestigial wing mutant treated with two concentration of *Mucuna pruriens* seed and *Glycyrrhiza glabra* root extract compared to control flies. In contrast to the results obtained from the present study on rat treated with monosodium glutamate have showed decreased locomotor activity due to the induction of neurotoxicity in rat and similar results were also shown in the rat treated with *Pongamma pinnata* significantly improved monosodium glutamate induced alteration in behavior and locomotor activity[18]. Rats treated with different concentration of Jessica has shown a significant reduction in locomotor activity when compared to control[19]. The *Mucuna pruriens* is rich in L- dopa, an enhancer of dopamanergic neurons hence the activity level of the flies has increased. Hence in the present study motor neuron activity is triggered more by *Mucuna pruriens* compared to *Glycyrrhiza glabra*. The peak activity levels are advanced in treated groups phase 19h in *Mucuna pruriens* in *D. melanogaster* in 22h in *Glycyrrhiza glabra* treated groups comparatively activity levels are less in vestigial wing mutant however it exhibits higher activity in treated groups. Similarly in *D. melanogaster*, vestigial wing mutants also exhibit greater efficiency in *Mucuna pruriens* treated groups compared to *Glycyrrhiza glabra*. Hence overall results show increase in locomotor activity proving enhancement in motor neuron activity.

Conclusion

Mucuna pruriens seed extract and *Glycyrrhiza glabra* root extract were the two plants selected to test their efficiency on the locomotor behaviour of *D. melanogaster* and vestigial wing mutant. Experimental stocks were reared in 22±1°C temperature with 75% relative humidity. After conducting the pilot experiment the doses were fixed mentioned it as T1 and T2 for both plants extract 2.0mg/100mL and 2.5mg/100mL for *Mucuna pruriens* seed extract and 1.0 mg/100mL and 2.0mg/100mL for *Glycyrrhiza glabra* root. Locomotor behaviour was assessed using the Activity Monitor (Trikinetics, UK). Increased locomotor activity was observed in both the plant extracts treated groups when compared to control group in *D. melanogaster* and vestigial wing mutant flies.

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Conflict of Interest

Authors declare no competing interests.

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